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Gender specific differences in neurodevelopmental effects of prenatal exposure to very low-lead levels: The prospective cohort study in three-year olds

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ABSTRACT

The primary purpose of this study was to assess the relationship between very low-level of prenatal lead exposure measured in the cord blood (<5 μg/dL) and possible gender-specific cognitive deficits in the course of the first three years of life. The accumulated lead dose in infants over the pregnancy period was measured by the cord blood lead level (BLL) and cognitive deficits were assessed by the Bayley Mental Development Index (MDI). The study sample consisted of 457 children born to non-smoking women living in the inner city and the outlying residential areas of Krakow. The relationship between prenatal lead exposure and MDI scores measured at 12, 24 and 36 months of age and adjusted to a set of important covariates (gender of child, maternal education, parity, breastfeeding, prenatal and postnatal environmental tobacco smoke) was evaluated with linear multivariate regression, and the Generalized Estimating Equations (GEE) longitudinal panel model. The median of lead level in cord blood was 1.21 μg/dL with the range of values from 0.44 to 4.60 μg/dL. Neither prenatal BLL (dichotomized by median) nor other covariates affected MDI score at 12 months of age. Subsequent testing of children at 24 months of age showed a borderline significant inverse association of lead exposure and mental function (beta coefficient = −2.42, 95%CI: −4.90 to 0.03), but the interaction term (BLL × male gender) was not significant. At 36 months, prenatal lead exposure was inversely and significantly associated with cognitive function in boys (Spearman correlation coefficient = −0.239, $p = 0.0007$) but not girls ($r = -0.058$, $p = 0.432$) and the interaction between BLL and male gender was significant (beta coefficient = −4.46; 95%CI: −8.28 to −0.63). Adjusted estimates of MDI deficit in boys at 36 months confirmed very strong negative impact of prenatal lead exposure (BLL > 1.67 μg/dL) compared with the lowest quartile of exposure (beta coefficient = −6.2, $p = 0.002$), but the effect in girls was insignificant (beta coefficient = −0.74, $p = 0.720$). The average deficit of cognitive function in the total sample over the first three years of life (GEE model) associated with higher prenatal lead exposure was also significant (beta coefficient = −3.00; 95%CI: −5.22 to −0.70). Beside prenatal lead exposure, presence of older siblings at home and prenatal environmental tobacco smoke had a negative impact on MDI score. Better maternal education showed a strong beneficial effect on the cognitive development of children. Conclusion: the study suggests that there might be no threshold for lead toxicity in children and provides evidence that 3-year old boys are more susceptible than girls to prenatal very low lead exposure. The results of the study should persuade policy makers to consider gender-related susceptibility to lead and possibly to other toxic hazards in setting environmental protection guidelines. To determine whether the cognitive deficit documented in this study persists to older ages, the follow-up of the children over the next several years is to be carried out.

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1. Introduction

Many epidemiologic studies have provided a solid basis to believe that prenatal exposure to low levels of lead may have demonstrable effects on cognitive and behavioral development of children [1–3]. Lead easily crosses biological membranes including placenta and the blood-brain barrier; and this may result in considerable deposition of lead in the fetal central nervous system. Because the fetal and infant brains are in a state of rapid growth, impairment of brain cognitive function may arise from the relatively minor prenatal toxic exposure.

Numerous cross-sectional and cohort studies have reported associations between exposure to environmental lead and adverse

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neurodevelopmental outcomes in children such as decreased motor function, behavior problems and lower scholastic achievements [4–11], but the effect of timing of exposure and the biological mechanisms underlying the associations have not been fully elucidated. Needleman et al. [12], in a meta-analysis of modern studies of childhood exposures to lead in relation to IQ, found strong support for the hypothesis that lead impairs children's IQ. However, there is a scarcity of cohort studies, which considered the effect of very low prenatal lead exposure on neurodevelopment of infants and very young children. Canfield et al [2], and Lanphear et al [3] have published meta analyses showing effects below 10 with an apparent greater risk at the lower level. In the present study the average lead concentration in cord blood was very low (median blood lead 1.21 μg/dL.

Up to now, in epidemiologic studies the gender-specific effects of prenatal lead exposure on neurocognitive development have not received adequate attention, and sex of the child in the statistical analysis of the epidemiologic data was mostly treated as a confounding variable. However, gender differences in toxicology begin at the gamete and embryo stage under genetic and hormonal control and affect xenobiotic exposures, metabolism, susceptibility, risk and health through development, maturation and in later life. Moreover, in earlier studies on the neurodevelopment of children in the context of prenatal lead toxicity, the role of important socio-cultural predictors was often underestimated or even not considered. However, the childbearing and postnatal child-rearing social environment reflects the most important social context of an infant, which has a strong impact on neurodevelopment of children.

The primary purpose of the study was to assess cognitive deficits in infants and young children in relation to very low-level prenatal exposure to lead and to examine gender-specific effects. The accumulated lead dose in infants over the pregnancy period was measured by the cord blood concentration of lead and the mental development of children was monitored at 12, 24 and 36 months of age by the Bayley test for Mental Development Index (MDI), which was designed for the assessment of cognitive development in early childhood. The secondary purpose of the study was to establish the relationship between fetal exposure and the timing of possible neurocognitive deficits over the first three years of life after adjustment for potential modifying or confounding effects of the quality of the childrearing environment (maternal education, older siblings, passive smoking).

2. Material and methods

The cohort originally consisted of 505 infants who were born at 29–42 weeks of gestation between January 2001 and February 2004 to mothers participating in an ongoing prospective cohort study. The current analysis covers 457 children for whom the database was complete. The design of this cohort prospective study and the selection of the population have been described previously [13]. Women attending ambulatory prenatal clinics in the first and second trimesters of pregnancy were eligible for the study. The enrollment included only non-smoking women with singleton pregnancies between the ages of 18–35 years, and who were free from chronic diseases such as diabetes and hypertension. Upon enrollment, a detailed questionnaire was administered to each subject to elicit information on demographic data, date of the last menstrual period (LMP), medical and reproductive history. Environmental tobacco smoke (ETS) in pregnancy was recorded during standardized interviews with women performed by trained interviewers at the second and thirds trimesters of pregnancy. Exposure was estimated using a series of questions on average number of cigarettes smoked at home or at work in the presence of women over the second and the third trimester of pregnancy. Postnatal ETS was categorized into 4 categories: 0. no exposure, 1.exposure present over 12 months, 2. present over 24 months, and 3. present over 36 months; breastfeeding was divided into two categories: 0.up to 6 months 1. breastfeeding longer than 6 months; variable older siblings categorized into three categories: 0. no older siblings, 1. one sibling, 2. two or more older siblings at home; maternal education was divided into three levels: 1. elementary, 2 secondary, 3 higher.

2.1. Blood sample collection and analysis

A cord blood sample (30–35 ml) was drawn into a vacutainer tube that had been treated with ethylene diamine tetra-acetate (EDTA). The tubes were inverted several times to mix the EDTA and the blood to prevent coagulation. Within 8 h of blood collection, the blood samples were transported to the clinical biochemistry laboratory at the University Hospital in Krakow for processing and storage. Packed red blood cells and plasma samples were separated and stored in liquid nitrogen in the laboratory prior to shipment to Columbia University. From Columbia University, portions of samples were then sent to the Centers for Disease Control (CDC) for chemical analysis. Blood samples for lead analysis were refrigerated without any processing. Whole blood lead concentrations were determined using inductively coupled plasma mass spectrometry CLIA'88 method "Blood lead cadmium mercury ICPMS_ITB001A". This multi-element analytical technique is based on quadrupole ICP-MS technology [14].

2.2. Mental developmental testing

The Bayley Scales of Infant Development – second edition (BSID-II) includes a mental scale, Mental Development Scale or Index (MDI) [15]. The BSID-II results are based on the assessment of 178 standardized activities. The number and sequence of these activities are chosen for each age group. The Mental Scale assesses items such as habituation, problem solving, early number concepts, generalization, classification, memory, vocalization, language and social skills.

The test results fall into 1 of 4 categories: 1) accelerated performance (equal or greater than the score of 115); 2) within normal limits (85–114); 3) mildly delayed performance (70–84); and 4) significantly delayed (equal or lower than 69). For the purpose of the statistical analysis, the first two categories (1 + 2) were combined into one group (the normal performance), and the other two groups (3 + 4) were treated as the group with delayed performance. The BSID-II test was administered to children within 4 weeks of the target age at the Department of Epidemiology and Preventive Medicine by five trained examiners, who were unaware of the child's exposure. Interpretation of the Bayley test was based on the detailed manual instructions for evaluators (Bayley Scales of Infant Development – Manual). Standardization of mental performance scoring was done within the team in the course of team practice session with the team leader (Ilona Lisowska–Miszczyk) who was trained at Columbia University with follow-up surveillance of the assessors by Dr J. Jankowski from Jeshiva University in New York.

2.3. Statistical data analysis

In the descriptive analysis, the distribution of various parameters related to women and newborns under study reflected by lead exposure level was considered. Chi-square statistics (nominal variables) and analysis of variance (numerical variables) tested differences between subgroups with lower and higher lead exposure. The relationship between lead in cord blood (dichotomized by median) and MDI scores was evaluated with linear multivariate regression. In order to assess the average effect of maternal lead exposure during pregnancy on the Bayley test scores measured at 12, 24 and 36 months of age, the Generalized Estimating Equations (GEE) model was also applied [16].

GEE utilizes data on all respondents, including those with incomplete protocols and permits simultaneous modeling of the relationship (regression) of specific risk factors with BSID II score and all three measurements over the follow-up.

GEE estimates regression coefficients taking into account the correlation between scores at ages 12, 24 and 36 months. All the models computed regression coefficients of the dependent variable (BSID-II performance scores) on the main predictor variable (lead cord blood level) and potential confounders or modifiers (maternal education, gender of child, parity, breastfeeding, prenatal and postnatal ETS). Data on breastfeeding practices were collected every three months though interviews with the mothers. Prenatal environmental tobacco smoke (ETS) was measured by a average number of cigarettes smoked daily in the presence of mother over pregnancy period and postnatal ETS by a number of cigarettes smoked daily at home in the presence of child over two years in postnatal period. In the statistical multivariate models we included the confounders, which in the univariate analysis were significantly associated with children cognitive development. Statistical analyses were performed with STATA 10 version software for Windows [17].

3. Results

The overall distribution of lead cord blood concentrations was skewed to the left (Fig. 1); the median value of the cord BLL was 1.21 μg/dL with a range from 0.44 to 4.60 μg/dL. In total, 25% of newborns had BLL concentrations above 1.67 μg/dL (95%CI: 1.60–1.80) and only 1% showed levels above 3 μg/dL (Fig. 1). The mean level of lead cord BLL in boys was did not differ from that observed in girls (1.23 vs.1.30 μg/dL).

Table 1 presents the characteristics of the children under study grouped by gender. Boys had higher values of birth outcome parameters (weight, length and head circumference), but showed significantly lower MDI scores at each of the follow-up time points. Neither maternal education, parity, nor prenatal or postnatal exposure to ETS differed across the gender groups.

BSID-II scores measured at the three follow-up time points correlated significantly with each other. The correlation coefficient between MDI scores measured at 12 months and 24 months was 0.38 (95%CI: 0.30–0.46) and that between MDI scores at 24 months and 36 months was 0.60 (95%CI: 0.54–0.66). The cognitive score of children over the 3-year follow-up increased with age. There were 6.3% children with delayed cognitive functions at 12 months of age; this proportion decreased to 4.7% by the age of 36 months.

On average, the mental function of girls was significantly higher than that of boys, and the gap between the MDI scores observed between boys and girls at 12 months persisted and even became a little wider by the age 36 months (Fig. 2).

Table 2 shows that MDI scores measured over the course of the follow-up and BLL only inversely correlated in boys at 36 months of age (Spearman rank correlation). Among boys there was a significant inverse trend of MDI score at age 36 months with the level of the lead cord blood (in quartiles) (Table 3). Among boys at age of 24 months the inverse trend was similar but at borderline significance (data not shown).

Tables 4–6 present the effects of prenatal lead exposure dichotomized by the median (1.21 μg/dL) on cognitive function (MDI), estimated by linear multiple regression models where the effects were adjusted for potential confounders (maternal education, parity, gender, duration of breastfeeding and prenatal and postnatal ETS exposure). The effect of prenatal lead exposure was neither significant on MDI score at 12 months of age nor on other covariates (Table 4). Subsequent testing of children at 24 months of age showed a borderline significant inverse association of lead exposure and mental function (beta coefficient = −2.42, 95%CI: −4.90 to 0.03), however the interaction term (cord BLL×gender) was insignificant (beta coefficient −0.50, $p = 0.84$). While we observed a positive association of MDI score with maternal education and parity, prenatal ETS had a

Table 1
Characteristics of the study subjects grouped by gender.

	Total $N = 457$	Gender		
		Boys $N = 234$	Girls $N = 223$	p
Maternal characteristics				
Mother's age				
Mean	27.50	27.19	27.83	
SD	3.52	3.61	3.40	0.0497
Education				
Elementary *n* (%)	43 (9.4%)	22 (9.4%)	21 (9.4%)	
Secondary *n* (%)	115 (25.2%)	58 (24.8%)	57 (25.6%)	0.9811
Higher *n* (%)	299 (65.4%)	154 (65.8%)	145 (65.0%)	
Infant characteristics				
Parity				
1 *n* (%)	289 (63.2%)	148 (63.2%)	141 (63.2%)	
≥2 *n* (%)	168 (36.8%)	86 (36.8%)	82 (36.8%)	1.0000
Gestational age (weeks): (29–42)				
Mean	39. 41	39.30	39.51	
SD	1.43	1.57	1.26	0.1197
Birth weight (g)				
Mean	3424.8	3494.4	3351.8	
SD	465.0	471.7	447.4	0.0010
Length at birth (cm)				
Mean	54.65	55.04	54.24	
SD	2.839	2.929	2.689	0.0026
Head circumference (cm)				
Mean	33.86	34.18	33.52	
SD	1.451	1.444	1.385	0.0000
Bayley–MDI12				
Mean	101.04	100.20	101.89	
SD	10.359	10.430	10.240	0.0897
Missing data – *n*	16	11	5	
Bayley–MDI24				
Mean	101.19	97.69	104.71	
SD	13.033	11.546	13.521	0.0000
Missing data – *n*	42	25	17	
Bayley–MDI36				
Mean	103.10	101.07	105.25	
SD	10.263	9.964	10.160	0.0001
Missing data – *n*	73	37	36	
Pb-cord blood (μg/dL)				
Mean	1.379	1.353	1.407	
SD	0.585	0.553	0.618	0.3217
Prenatal ETS exposure				
No *n* (%)	337 (73.7%)	172 (73.5%)	165 (74.0%)	
Yes *n* (%)	120 (26.3%)	62 (26.5%)	58 (26.0%)	0.9905
Postnatal ETS exposure				
No exposure *n* (%)	318 (80.5%)	159 (78.7%)	159 (82.4%)	
Up to 12 months *n* (%)	24 (6.1%)	16 (7.9%)	8 (4.1%)	0.3232
13–24 months *n* (%)	22 (5.6%)	13 (6.4%)	9 (4.7%)	
More than 24 months *n* (%)	31 (7.8%)	14 (6.9%)	17 (8.8%)	
Missing data – *n*	62	32	30	

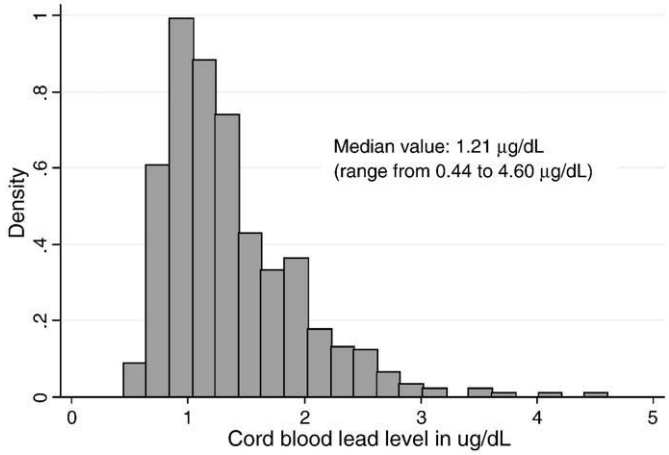

Fig. 1. Histogram of BLL in the total study sample.

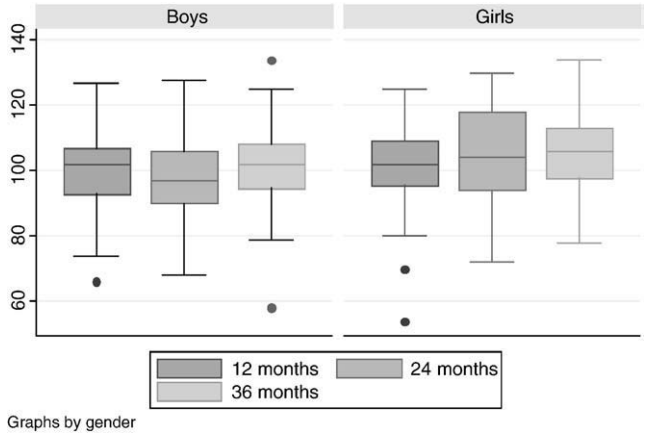

Fig. 2. Box plot of MDI scores over the follow-up groped by gender. Kruskal–Wallis test for differences in MDI scores between gender groups measured at different time points: MDI_{12} $Chi^2 = 4.045$, 1 *df*, $p = 0.044$. MDI_{24} $Chi^2 = 30.172$, 1 *df*, $p < 0.001$. MDI_{36} $Chi^2 = 15.026$, 1 *df*, $p < 0.001$.

significant negative impact on the neurocognitive function of children at 24 months of age (Table 5).

At 36 months of age, we observed a significant interaction between prenatal BLL and MDI score in boys (beta coefficient = −4.46; 95%CI: −8.28 – 0.63). In adjusted models, each μg/dL increase of the cord BLL was significantly associated with boys having about 1 less point on Bayley's MDI score. As at the 24 months of age, there was a strong positive effect of higher maternal education on MDI score (beta coefficient = 4.82; 95%CI: 1.08–8.56). The negative effect of prenatal ETS on MDI score was again confirmed (beta coefficient = −3.24; 95% CI: −6. 11 to −0.37) and the presence of older siblings at home correlated inversely with the cognitive development measured at 36 months of age (Table 6). Regression of fitted MDI_{36} score by gender on log-transformed cord blood lead levels are presented in Fig. 3, and the comparison of the distributions of MDI_{36} scores between gender groups and the prenatal lead exposure level (BLL dichotomized by median) have been shown in Fig. 4.

In Table 7, we presented adjusted estimates of MDI deficit at 36 months of age due to prenatal lead level (in quartiles) based on multiple linear regression models by gender strata. While there was very strong negative impact of prenatal lead exposure (BLL > 1.67 μg/dL) in boys (beta coefficient = −6.2, $p = 0.002$), the effect in girls was insignificant (beta coefficient = −0.74, $p = 0.720$).

The longitudinal analysis of the association between prenatal lead exposure (dichotomized by median BLL) and cognitive function in total study sample (GEE model) is presented in Table 8. This analysis again showed that the average adjusted deficit in the cognitive development in the total sample over the first three years of life associated with higher prenatal lead exposure (BBL > 1.67 μg/dL) compared with the lowest quartile of exposure (BLL < 0.99 μg/dL) was also significant (beta coefficient = −3.00; 95%CI: −5.22 to −0.70), but the interaction term (BLL × gender) was insignificant (beta coefficient −1.79; $p = 0.270$). The adjusted effect of prenatal ETS exposure remained inversely associated with the MDI score (beta coefficient = −2.17, 95%CI: −4.01 to −0.34). The mental function of boys was significantly lower than in girls (beta coefficient = −4.11; 95%CI: −5.66 to −2.58) and the impact of maternal education

Table 2
Spearman rank correlation coefficients between lead cord blood concentrations (μg/dL) and BSIDII scores measured at 12, 24 and 36 months of age grouped by gender.

	MDI_{12}	MDI_{24}	MDI_{36}
Total sample	−0.073 ($p = 0.127$)	−0.107 ($p = 0.028$)	−0.144 ($p = 0.005$)
Boys	−0.078 ($p = 0.245$)	−0.126 ($p = 0.068$)	−0.239 ($p = 0.001$)
Girls	−0.111 ($p = 0.115$)	−0.110 ($p = 0.115$)	−0.058 ($p = 0.432$)

Table 3
Cross-tabulation of mean BSIDII mental score at 36 months of age grouped by the lead cord blood level (in quartiles).

Lead cord blood level (μg/dL)	Number of observations	Mean	SD	Analysis of variance for trend
Boys				
≤0.99	59	106.222	9.407	$F = 6.64$
1.00–1.21	59	100.889	11.406	*d.f.* = 3, 194
1.22–1.67	64	97.8491	7.463	$p = 0.0003$
>1.67	53	99.8696	9.389	
Girls				
≤0.99	56	106.457	8.878	$F = 0.37$
1.00 – 1.21	55	104.304	10.127	*d.f.* = 3, 184
1.22–1.67	51	104.953	10.783	$p = 0.7771$
>1.67	62	104.943	10.953	

Table 4
Multiple linear regression models testing prenatal lead effect at 12 months of age, (MDI scores adjusted for potential confounders).

Predictors	Coefficient	p>t	95% confidence interval	
Maternal education				
Elementary	Reference			
Secondary	−0.369	0.858	−4.412	3.674
_Higher	1.808	0.365	−2.116	5.732
Older siblings				
No older siblings	Reference			
One older sibling	0.881	0.446	−1.390	3.152
Two or more older siblings	−0.892	0.733	−6.029	4.245
Breastfeeding	1.038	0.388	−1.324	3.401
Cord BLL>1.21 μg/dL	−0.259	0.864	−3.226	2.708
Gender	−1.533	0.295	−4.409	1.343
Interaction term BLL × gender (boys)	−0.304	0.885	−4.422	3.814
Prenatal ETS	−0.660	0.675	−0.377	0.244
Postnatal ETS	0.224	0.591	−0.594	1.042
Constans	99.6937	0.000	95.104	104.283

Maternal education: 1. elementary, 2 secondary, 3 higher. Older siblings: 0. no older siblings, 1. one sibling, 2. two or more older siblings at home. Breastfeeding: 0. up to 6 months 1. longer than 6 months; Gender: 0. females, 1.males. Prenatal ETS: 0 no exposure, 1 exposure present over the second and the third trimester of pregnancy. Postnatal ETS: 0.no exposure, 1. exposure over 12 months, 2. exposure over 24 months, 3. over 36 months.

Table 5
Multiple linear regression models testing prenatal lead effect at 24 months of age, (MDI scores adjusted for potential confounders).

Predictors	Coefficient	*p>t*	95% confidence interval	
Maternal education				
Elementary	Reference			
_Secondary	0.999	0.683	−3.809	5.808
_Higher	3.706	0.119	−0.964	8.375
Older siblings				
No older siblings	Reference			
One older sibling	−1.228	0.371	−3.924	1.469
_Two or more older siblings	−7.757	0.012	−13.793	−1.721
Breastfeeding>6 months	1.901	0.183	−0.903	4.705
Cord BLL>1.21 μg/dL	−2.225	0.212	−5.728	1.278
Boys	−6.440	0.000	−9.840	−3.039
Interaction term BLL × gender (boys)	−0.500	0.840	−5.375	4.375
Prenatal ETS	−4.430	0.021	−7.960	−0.650
Postnatal ETS	0.129	0.793	−0.834	1.092
Constants	103.194	0.000	97.747	108.640

Maternal education: 1. elementary, 2 secondary, 3 higher. Older siblings: 0. no older siblings, 1. one sibling, 2. two or more older siblings at home. Breastfeeding: 0. up to 6 months 1. longer than 6 months. Gender: 0. females, 1.males. Prenatal ETS: 0 no exposure, 1 exposure present over the second and the third trimester of pregnancy. Postnatal ETS: 0. no exposure, 1. exposure over 12 months, 2. exposure over 24 months, 3. over 36 months.

Table 6
Multiple linear regression models testing prenatal lead effect at 36 months of age, (MDI scores adjusted for potential confounders).

Predictors	Coefficient	$p>t$	95% confidence interval	
Maternal education				
Elementary	Reference			
_Secondary	1.403	0.472	−2.433	5.239
_High	4.819	0.012	1.084	8.555
Older siblings				
No older siblings	Reference			
_One older sibling	−1.786	0.100	−3.914	0.342
Two or more older siblings	−8.612	0.000	−13.325	−3.898
Breastfeeding>6 months	2.486	0.026	0.295	4.677
Cord BLL>1.21 μg/dL	0.245	0.862	−2.517	3.007
Boys	−1.983	0.146	−4.659	0.693
Interaction term BLL×gender (boys)	−4.456	0.023	−8.283	−0.628
Prenatal ETS	−3.240	0.027	−6. 110	−0.370
Postnatal ETS	0.384	0.317	−0.370	1.138
_cons	101.052	0.000	96.726	105.378

Maternal education: 1. elementary, 2 secondary, 3 higher. Older siblings: 0. no older siblings, 1. one sibling, 2. two or more older siblings at home. Breastfeeding: 0. up to 6 months 1. longer than 6 months. Gender: 0. females, 1.males. Prenatal ETS: 0 no exposure, 1 exposure present over the second and the third trimester of pregnancy. Postnatal ETS: 0. no exposure, 1. exposure over 12 months, 2. exposure over 24 months, 3. over 36 months.

remained strongly positive. Interestingly, presence of older siblings at home had a significant negative impact on cognitive function of children, and the beneficial effect of breastfeeding was at the border significance level.

4. Discussion

The infants in our study were exposed prenatally to very low lead concentrations, that ranged between 0.44–4.60 μg/dL with a median 1.21 μg/dL. On average, MDI scores over 3-year follow-up were inversely and significantly correlated with lead cord blood concentrations (GEE model). The effects of prenatal lead exposure on the mental score in girls were neither confirmed at each time point of the follow-up nor in the longitudinal GEE model. The negative impact of prenatal lead exposure (above>1.21 μg/dL) on MDI score observed only in boys at age of 3 years amounted to a deficit of 4.5 points (between the high and low exposed, as determined by a cut-off point at the median). Only at age of three years there was a statistically significant interaction term (BLL×male gender) and a significant trend of MDI deficit with prenatal lead exposure (in quartiles of exposure). In all linear multivariate and GEE models, the association between cord blood lead level and the cognitive scores of children was adjusted for potential confounders such as maternal education, gender of child, parity, breastfeeding and ETS exposure.

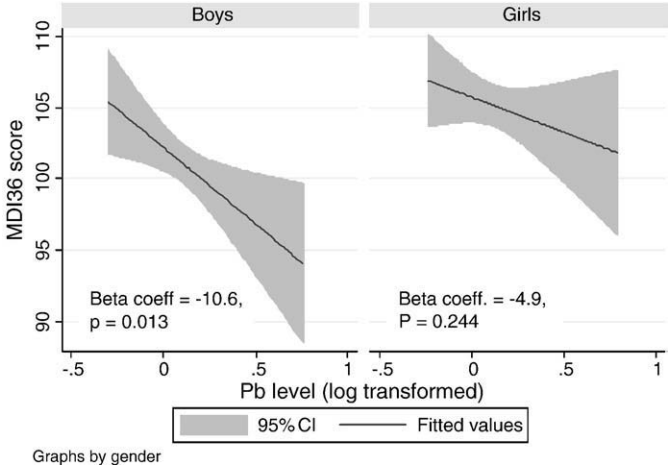

Fig. 3. Regression of fitted MID_{36} score on log-transformed concentrations of cord blood lead level grouped by gender.

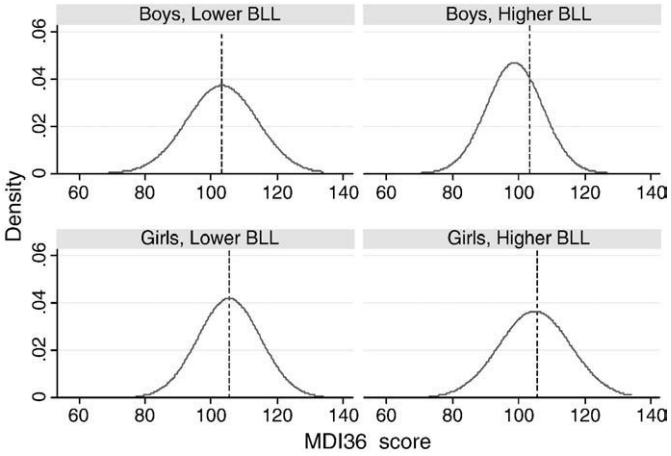

Fig. 4. Comparison of the distributions of MDI_{36} scores between gender groups and the prenatal lead exposure level (BLL dichotomized by median). Vertical reference lines correspond to mean MDI_{36} score in boys and girls found in the lower exposed group.

On average, the mental function of girls was significantly higher than that of boys and the initial gap between the MDI scores observed between boys and girls at 12 months persisted and even became a little wider by the age 24 months The cognitive gap between boys and girls might reflect different rates in cognitive development in boys and girls in early childhood or result from different effects of escalating socio-cultural challenges to children's cognitive ability with age. The cognitive gap might also reflect a possible delay in the distinct manifestation of the cognitive deficit recognizable by BSIDII test or the persisting functional brain damage in boys in the sphere of cognitive function. The results also indicated that other early life factors such as social and maternal behavior or maternal education may modulate the associations between prenatal lead exposure and cognitive development.

Early cognitive deficits in early childhood related to very low prenatal lead exposure are biologically plausible since the peak vulnerability of children to environmental lead would correspond to fetal rapid brain growth, especially during times of synaptogenesis, arborization, and dendritic pruning [18–20]. The proposed biological

Table 7
Multiple linear regression models testing prenatal lead level (in quartiles*) at 36 months of age by gender strata, (MDI scores adjusted for potential confounders).

	Coefficient	t	$p>t$	95% confidence interval	
Total					
Reference BLL level≤0.99 μg/dL (1st quartile)					
BLL 1.00–1.21 μg/dL	−3.494	−.2.53	0.012	−6.214	−0.775
BLL_1.22–1.67 μg/dL	−4.459	−3.17	0.002	−7.223	−1.694
BLL_>1.67 μg/dL	−3.163	−2.26	0.024	−5.914	−0.412
Boys					
Reference BLL level≤0.99 μg/dL (1st quartile)					
BLL 1.00–1.21 μg/L	−5.451	−2.98	0.003	−9.064	−1.839
BLL_1.22–1.67 μg/dL	−8.373	−4.55	0.000	−12.006	−4.741
BLL_>1.67 μg/L	−6.158	−3.21	0.002	−9.946	−2.370
Girls					
Reference BLL level≤0.99 μg/dL (1st quartile)					
BLL 1.00–1.21 μg/dL	−1.810	−0.87	0.384	−5.905	2.287
BLL 1.22–1.67 μg/dL	−0.040	−0.02	0.985	−4.284	4.203
BLL>1.67 μg/dL	−0.738	−0.36	0.720	−4.796	3.319

Table 8
Cognitive development of children over the follow-up in the GEE models.

Predictors	Coefficient	$p>z$	95% confidence interval	
Maternal education				
Elementary	Reference			
Secondary	0.86	0.581	−2.19	3.90
Higher	3.75	0.011	0.85	6.64
Breastfeeding>6 months	1.49	0.099	−0.28	3.26
Boys	−4.11	0.000	−5.66	−2.58
Older siblings				
No older siblings	Reference			
One older sibling	−0.81	0.349	−2.51	0.89
Two or more older siblings	−4.52	0.020	−8.33	−0.71
BLL in quartiles				
Reference BLL level≤0.99 μg/dL (1st quartile)				
BLL 1.00–1.21 μg/dL	−1.81	0.100	−3.98	0.35
BLL 1.22–1.67 μg/dL	−2.88	0.009	−5.04	−0.71
BLL>1.67 μg/dL	−3.00	0.010	−5.22	−0.70
Prenatal ETS	−2.17	0.020	−4.01	−0.34
Postnatal ETS	0.23	0.469	−0.40	0.87
Constants	107.13	0.000	102.92	111.35

Maternal education: 1. elementary, 2 secondary, 3 higher. Older siblings: 0. no older siblings, 1. one sibling, 2. two or more older siblings at home. Breastfeeding: 0. up to 6 months 1. longer than 6 months. Gender: 0. females, 1.males. Prenatal ETS: 0 no exposure, 1 exposure present over the second and the third trimester of pregnancy. Postnatal ETS: 0.no exposure, 1. exposure over 12 months, 2. exposure over 24 months, 3. over 36 months.

mechanism of lead toxicity are based on the effects of lead on the blood-brain barrier and on neurotransmitter function and hypothesize that lead produces 'synaptic noise' and inappropriate pruning of the synaptic connections.

Most epidemiologic studies have reported neurobehavioral effects of lead at higher exposures than those reported here, either occurring in childhood or prenatally. The meta-analysis of 12 studies of childhood exposure to lead in relation to IQ found that the estimated deficits of approximately 2–6 points on tests of intelligence for every 10 μg/dl increase in blood lead concentrations among school children [12]. However, the power of the studies to find an effect was limited, below 0.6 in 7 of the 12 studies. Our results are consistent with the data reported by Bellinger et al [21] who analyzed longitudinally the effect of prenatal and postnatal lead exposure on early cognitive development of children (BSID) examined semiannually over two years. In their work, the estimated difference between the adjusted mental performance of the prenatal lower exposure group (<3.0 μg/dL) and the higher exposure group (>10 μg/dL) was 4.8 points. Scores were not related to infants' postnatal blood lead levels.

Gender differences in exposure to toxic metals have been well-documented in the literature, but not much about gender differences in susceptibility. It is thought that gender differences in susceptibility to toxic environment may result from the already well-documented fact that specific areas of the brain develop differently in males and females under the influence of a disproportionate number of genes present in the X and Y chromosomes and sex hormones [22]. It is understood that estrogen plays an important role in regulating neural structure and brain function and there is a distinct anatomical difference in the distribution and density of estrogen receptors in the brain between males and females. As males generally have fewer estrogen receptors throughout the CNS as compared to females, it is thought that the consequences of neurotoxicant exposure and the gender differences in the response to toxic exposure can partially depend on the protective effects of estrogen. Moreover, the CNS maturation is accompanied by extensive pruning and competitive eliminations, leading to restructuring and reorganization of brain function. This in turn may lead to more distinct and permanent gender-related differences in the various neurotransmitter systems in both health and disease. These differences may be manifested in early cognitive responses to xenobiotics in childhood.

The results of our study strengthen evidence that cognitive deficits of boys may reflect gender-specific differences in the various neurotransmitter systems, which subsequently lead to increased susceptibility. Up to now few studies in humans have found any sizeable gender differences following developmental exposure to very low levels of environmental toxic metals. However, Bellinger and colleagues [1,21], in their studies of lead exposure at levels higher than those in the present study, found that male gender was more affected and childhood social class mitigate the adverse association of lead exposure and childhood intelligence. Grandjean et al [23] in the Faroe Island mercury study found that schoolboys in the more exposed group showed worse reaction time and hand-eye coordination than controls.

In the course of our cohort study we have been able to show not only the adverse effect of lead exposure but also prenatal ETS exposure on the cognitive development over 3 year follow-up. The adverse impact of fetal exposure to ETS over the first years of life may be due to mechanisms exerted by ETS constituents. They may alter receptor-mediated cell signaling in the brain [24], induction of P450 enzymes [25] or cause DNA damage by activating apoptotic pathways [26,27]. Our observation is in agreement with the results of Rauh et al. [28] who reported significant decrease in cognitive test scores among two-year olds who were exposed prenatally to ETS, after controlling for ETS exposure during the first two postnatal years. Since indoor PAHs result to a great extent from ETS, the ETS-related cognitive deficit could be attributable in part to PAHs. The earlier study of Perera et al. [29] showed that PAHs at levels encountered in the urban community may adversely affect cognitive development of preschool children. It is not yet clear to which extent the higher levels of maternal blood-lead observed in ETS-exposed women may eventually explain ETS-related cognitive deficits in very young children [30].

At present there is no definitive and clear explanation for the positive association between maternal education and neurocognitive development of children, which has been observed in our study. Educational level of mothers is not only a proxy of socioeconomic status of the family, but it may be related to other relevant factors such as maternal behavior, life style, dietary habits before and during pregnancy or feeding practices of infants and young children. With the exception of breastfeeding, none of these variables mentioned were considered in our analysis. Maybe less educated mothers are not as responsive as better educated mothers to their infants' needs or present some less favorable behavior during early childhood. Children living in low socio-economic environment are more likely to be exposed to lead or other chemical hazards and the adverse effects of lead may be more pronounced in lower compared to higher socio-economic groups. Studies carried out by Bellinger et al. [31] and Winneke et al. [32] suggest that social context modifies the effects of chemical neurotoxins. For example, material hardship has been demonstrated to modify the neurotoxic effects of tobacco smoke in children in the study done by Rauh et al [28]. The way in which maternal behavior may affect the development of children was discussed in the recently published paper by Surkan et al [33]. The authors explored the modifying effects of maternal self-esteem on the association between exposure to lead and neurodevelopment of children. In adjusted models, each point increase in maternal self-esteem was significantly associated with children having 0.2 higher score on the Bayley's MDI score. The observed beneficial effect of self-esteem was independent of child gender, blood lead levels, maternal IQ, maternal education, parity, maternal smoking during pregnancy, and alcohol consumption. Moreover, there was evidence that maternal self-esteem attenuated the negative effects of lead exposure, although the interaction term was insignificant. With respect to behavioral pathways, higher self-esteem may be linked to a variety of positive health practices. One can speculate that mothers with higher self-esteem may be less depressed, have a better mother–child

interaction and provide a higher level of intellectual stimulation or emotional support to their children.

Interestingly, the presence of older siblings at home showed a negative impact on mental development of children in our study. The number of children at home may again be a proxy not only for socio-environmental factors operating in early childhood, but also related to a higher risk of viral infections introduced by older siblings into household, which in turn may effect children's neurodevelopment. On the other hand, maternal attention being spread over a couple of children may mean mothers are less responsive to each child's particular needs. In this respect the results of our study calls for more research efforts aiming at explaining the other factors hidden behind proxy measures for the quality of maternal care of babies.

The most sensitive neurocognitive endpoints for low-level lead toxicity are not yet known. Some investigators have focused on motor development and others on visual/motor performance based upon the effects of lead observed in occupational settings. In order to capture the early cognitive outcomes of lead exposure in the womb, we have chosen the Bayley mental test for measuring the mental function at follow-up, which is a well standardized tool suitable for intellectual deficits among infants and very young children longitudinally. Not only are the scales well standardized, but they offer an early and fairly comprehensive measure of cognitive functioning. The Bayley taps abilities such as attention, memory, and perceptual reasoning which are thought to be fundamental components of early as well as later intellectual functioning.

Because many epidemiologic studies involve small study samples, the lack of the power has been a major problem in human studies. Ours was adequate to detect the significance of effect of lead among boys. The strengths of the study are that the design was balanced in terms of sample size of boys and girls, and that a set of important confounders potentially affecting child development such as maternal education and ETS exposure was considered. However, our study sample may not be representative of the entire female urban population in the country because enrollment covered only pregnant nonsmoking women with singleton pregnancies between the ages of 18 and 35 years who were free from such chronic diseases as diabetes and hypertension. On the other hand, these inclusion criteria helped us eliminate from the study infants who were at a greater risk for neurocognitive disorders because of maternal chronic diseases or active smoking. Nevertheless, we were not able to control the cognitive function of children for postnatal lead exposure, but the postnatal exposure might not confound the effect of prenatal lead exposure as it was shown by Bellinger et al. [21]. Neither paternal occupational exposure as a possible source of postnatal lead exposure nor various behavioral features of boys and girls (e.g. child's playing outdoors, increased hand to mouth activity, etc.) which could have affected postnatal exposure to lead were considered in the study. However, we were able to confirm that neither the communal water supply system nor domestic heating, nor vicinity to busy roads differentiated the exposure groups defined by the prenatal BLL.

Summing up, the data from our study demonstrated a neurotoxic impact of very low-level of prenatal lead exposure in male children. The results provide evidence that susceptibility to prenatal lead exposure is different across gender groups and should persuade policy makers to take into consideration gender-related susceptibility in setting environmental protection guidelines. To determine whether the cognitive deficit documented in this study persists to older ages, we plan to continue the follow-up of the study sample over the next several years. This will enable us to assess the dynamics of mental development of children in relation to prenatal and postnatal lead exposure.

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